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Growth of Male-Specific Bacteriophage in *Proteus mirabilis* Harboring F-Genotes Derived from *Escherichia coli*

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ABSTRACT

HORIUCHI, KENSUKE (Yale University, New Haven, Conn.), AND EDWARD A. ADELBERG. Growth of male-specific bacteriophage in *Proteus mirabilis* harboring F-genotes derived from *Escherichia coli*. J. Bacteriol. **89**:1231–1236. 1965.—Male-specific bacteriophage MS2 was shown to infect and grow in *Proteus mirabilis* strains which harbor F-genotes derived from *Escherichia coli* K-12. The burst size was 2,000 to 3,000, which is similar to that in *E. coli* K-12, whereas the latent period was 45 min, definitely longer than that in *E. coli*. In spite of the multiplication of MS2 in male *P. mirabilis* in broth, *P. mirabilis* strains failed to show plaque formation by MS2 on agar plates; this failure may be related to the low efficiency of phage adsorption. No host-controlled modification of MS2 by *P. mirabilis* was detected.

It is known that, in Hfr populations of *Escherichia coli* K-12, F′ cells occur in which the sex factor (F) has returned to the autonomous state and has incorporated a fragment of the chromosome (Jacob and Adelberg, 1959; Adelberg and Burns, 1960). This autonomous element, which has been called an F-genote, can be transferred to recipient strains at high frequency.

It has been reported that the F-genotes F-*lac* and F_{13} of *E. coli* K-12 can be transferred to strains of *Proteus*, in which the guanine plus cytosine (GC) content of the deoxyribonucleic acid (DNA) is quite different from that found in *E. coli* (Falkow et al., 1964). We have also observed that two other F-genotes, F_{14}, which harbors a chromosomal segment containing the loci *ilv*, *arg*, and *met* (Pittard, Loutit, and Adelberg, 1963), and F_{16}, which carries only the *ilv* loci (Pittard and Adelberg, 1963), can be transferred to *Proteus mirabilis* from *E. coli* K-12. (The symbols refer to genetic loci governing the synthesis of enzymes of certain biosynthetic pathways, as follows: *ilv*, isoleucine-valine; *arg*, arginine; *met*, methionine.) In the *P. mirabilis* F′ cells thus obtained, not only the genes on the chromosomal segment of the F-genote but also the sex factor itself seems to be functional, for the F-linked chromosomal genes can be transferred from the *P. mirabilis* strains back to *E. coli* recipient strains and can be eliminated by treatment with acridine orange, in agreement with the data of Falkow et al. (1964).

A number of phages have been described which can infect only male strains of *E. coli*, that is, strains harboring F. This specificity reflects the requirement of these phages for adsorption sites found only on male bacteria. Since *Proteus* can harbor F-genotes and other plasmids, e.g., P-*lac* (Wohlheiter et al., 1964), it seemed of interest to discover whether the male-specific bacteriophage, MS2, can infect and grow in such *Proteus* strains. The present paper reports that MS2 can multiply in *P. mirabilis* strains which carry F-genotes derived from *E. coli*.

MATERIALS AND METHODS

The media were those described by Adelberg and Burns (1960). The bacterial strains used are shown in Table 1. *P. mirabilis* F′ strains were obtained by mixing log-phase cells of an F′ strain of *E. coli* with log-phase cells of an auxotrophic strain of *P. mirabilis* at 37 C in broth. After incubation for 3 hr, the mixture was plated on minimal agar plates selective for *P. mirabilis* carrying one or more F-genote markers, and the colonies formed were purified by repeating single-colony isolations more than seven times. The frequency of recombination was dependent on the F-genote. In the case of F_{16}, for instance, the frequency of transfer (relative to donor cell input) was 10^{-3} from *E. coli* to *P. mirabilis*, 10^{-4} from *P. mirabilis* to *E. coli*, less than 10^{-9} from *P.*

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TABLE 1. *List of strains**

Species	Strain no.	Previous no.	Characters									Plasmids	Parent strains
			Nic	Ilv	Met	Ade	Arg	His	Pro	Lac	Str		
Proteus mirabilis	AC2505	PM1	−	+	+	+	+	+	+	−	S	—	—†
	AC2626	—	−	−	+	+	+	+	+	−	S	—	AC2505
	AC2634	—	−	+/−	+	+	+	+	+	−	S	F_{14}	AB1206 × AB2626
	AC2632	—	−	−	−	+	+	+	+	−	S	—	AC2626
	AC2636	—	−	+/−	−	+	+	+	+	−	S	F_{16}	AB1528 × AC2632
	AC2637	—	−	−	+	−	+	+	+	−	S	—	AC2626
	AC2638	—	−	+/−	+	−	+	+	+	−	S	F_{16}	AB1528 × AC2637
	AC2639	PM1F-*lac*	−	+	+	+	+	+	+	+/−	S	F-*lac*	—‡
	AC2640	PM1P-*lac*	−	+	+	+	+	+	+	+/−	S	P-*lac*	—‡
Escherichia coli (K-12)	AB1450	—	+	−	−	+	−	−	+	−	R	—	—
	AB1206	—	+	+	+	+	+	−	−	−	R	F_{14}	—
	AB1528	—	+	+/−	+	+	−	−	−	−	S	F_{16}	—
	AB2721	—	+	−	−	+	−	−	+	+/−	R	P-*lac*	AB2640 × AB1450
	AB259	—	+	+	+	+	+	+	+	+	S	F_1-integrated (Hfr)	—

* The following abbreviations are used: Nic, nicotinic acid; Ilv, isoleucine and valine; Met, methionine; Ade, adenine; Arg, arginine; His, histidine; Pro, proline; Lac, lactose; Str, streptomycin; R, resistant; S, sensitive; +/−, heterozygous.

† Provided by L. S. Baron.

‡ Provided by S. Falkow.

mirabilis to *P. mirabilis*, and 1 from *E. coli* to *E. coli*.

Antiserum against MS2, provided by A. J. Clark, was used for the neutralization of free phage in certain experiments. A dilution of the antiserum having a final K value of 4 min^{-1} was used, and neutralization was carried out for 5 min. Under these conditions, a survival of 0.2×10^{-8} is expected.

Lysates of MS2 were prepared by growing on *E. coli* strain AB259, unless otherwise noted. The titration of MS2 was carried out by plating the sample on nutrient plates using *E. coli* strain AB259 as the indicator, unless otherwise noted.

RESULTS

Preliminary experiments. To test several *P. mirabilis* strains for their ability to allow the growth of bacteriophage MS2, MS2 was added to broth inoculated with *P. mirabilis* cells. After standing at 37 C overnight, the cultures were treated with chloroform and plated on nutrient agar plates using *E. coli* strain AB259 as the indicator. The results shown in Table 2 indicate that the titer of MS2 increased from 10^2 to 10^5 times during the incubation with *P. mirabilis* F′ strains, whereas no increase of MS2 was observed in tubes with *P. mirabilis* F^- strains. On the other hand, strains of *P. mirabilis* or *E. coli* harboring the plasmid P-*lac* were found not to allow the multiplication of MS2.

Failure of MS2 to form plaques on P. mirabilis. Whereas *E. coli* strain AB1528, the F′ parent of *P. mirabilis* strains AC2636 and AC2638, allows MS2 to form plaques at the same efficiency as AB259, all attempts to produce MS2 plaques on AC2636 or AC2638 were unsuccessful. *P. mirabilis* cells swarm on nutrient agar but not on minimal agar; MS2 failed to form plaques on *P. mirabilis* on either medium, whereas it forms plaques on *E. coli* male strains with equal efficiency on minimal agar and on nutrient agar. When various numbers of MS2 particles (from 1 to 10^7 plaque-forming units) grown either on *E. coli* AB259 or on *P. mirabilis* AC2636 were spotted on supplemented minimal agar plates seeded with various numbers of *P. mirabilis* AC2636 cells (2×10^4 to 2×10^8 cells per plate), no lysis was observed.

Two possible reasons for the failure of MS2 to form plaques on *P. mirabilis* F′ strains were considered. One is that only a small portion of the *P. mirabilis* F′ population allows the growth of MS2, and the other is that MS2 is liberated from *P. mirabilis* cells without causing lysis.

Low efficiency of infection of male P. mirabilis by MS2. The efficiency of infection was estimated by plating for infective centers. Log-phase cultures of bacteria were mixed with MS2 in broth and incubated at 37 C for 5 or 15 min. After removal of free phage by treatment with anti-MS2 serum, the mixture was diluted and plated

TABLE 2. *Reproduction of MS2 in Proteus mirabilis F′ strains**

Bacteria added	Plasmids in the bacteria	Final titer of MS2†	Multiplication of MS2
None	—	1.7×10^3	0.6
AC2505	—	1.9×10^3	0.7
AC2637	—	1.8×10^3	0.7
AC2639	F-*lac*	3.8×10^8	1.4×10^5
AC2640	P-*lac*	1.6×10^3	0.6
AC2638	F_{16}	1.8×10^5	67
AC2636	F_{16}	7.2×10^8	2.7×10^5
AC2634	F_{14}	1.2×10^6	4.4×10^2
AB1450 (*Escherichia coli*)	—	1.7×10^3	0.6
AB2721 (*Escherichia coli*)	P-*lac*	1.7×10^3	0.6
AB259 (*Escherichia coli*)	F_1-integrated (Hfr)	1.5×10^{11}	5.6×10^7

* An amount (3 ml) of broth was inoculated with 0.1 ml of an overnight broth culture of the bacterial strain indicated, and with 0.1 ml of an MS2 lysate containing 8.7×10^3 plaque-forming units. Thus, the initial number of MS2 added to each tube was 2.7×10^3/ml. After incubation at 37 C for 12 hr, the cultures were treated with chloroform and plated on nutrient agar with *E. coli* AB259 as the indicator strain.

† Plaque-forming units per milliliter.

TABLE 3. *Efficiency of formation of infective centers**

MS2 grown on	Bacteria used for adsorption	Time of contact	Multiplicity	No. of infective centers as per cent of bacteria added	No. of infective centers as per cent of MS2 added
		min			
Escherichia coli AB259	AC2636	15	670	9.3	—
Escherichia coli AB259	AB259	15	290	140	—
Escherichia coli AB259	AC2636	5	0.03	—	2.1
Escherichia coli AB259	AB259	5	0.02	—	49
Proteus mirabilis AC2636	AC2636	5	0.04	—	1.2
Proteus mirabilis AC2636	AB259	5	0.03	—	53

* The experimental procedure is given in the text. All infective centers were assayed on *E. coli* AB259.

on nutrient agar with cells of *E. coli* strain AB259 to score the number of infective centers. As a control, anti-MS2 serum mixed with a phage suspension to which sterile broth had been added instead of bacteria; incubation, serial dilution, and plating in parallel with the experimental mixtures showed no detectable free phage. The results (Table 3) show that even at a multiplicity of infection as high as 670 less than 10% of the cells of *P. mirabilis* AC2636 could form infective centers, while 140% of an *E. coli* AB259 population gave infective centers under similar conditions (the value above 100% is due to the growth of the cells during incubation). At a lower multiplicity also, fewer infective centers were formed on *P. mirabilis* AC2636 than on *E. coli* AB259 (Table 3).

That the lower efficiency of infection of *P. mirabilis* with MS2 is due to poor adsorption of the phage was indicated by the following experiment. A 1-ml amount of a prewarmed suspension of MS2 was added to 1 ml of a log-phase culture of bacteria in broth at 37 C. The final concentration of phage was 4.8×10^7 particles per milliliter; the bacteria were in 5- to 10-fold excess. After 15 min of incubation at 37 C, the mixture was treated with chloroform and plated on *E. coli* AB259 to score free phage. The adsorption of MS2 by the male strain of *E. coli*, AB259, was found to be 71% under these conditions. The absorption of MS2 by a female strain of *E. coli*, or by an F′ strain of *P. mirabilis*, was not detectable by the difference in plaque count before and after incubation.

Assuming a lower limit of detection by differential count of 10%, we can only say that less than 10% of the phage particles were adsorbed by *P. mirabilis*. Up to 2% of the phage particles, however, yielded infective centers (Table 3, last column). Thus, a minimum of 20% of the adsorbed phage gave rise to productive infections.

Some (less than 10%) of the infective centers gave plaques with a bacterial colony at the center of the plaque. These colonies were isolated and

shown to consist of *P. mirabilis* F′ cells, which indicates that many male *P. mirabilis* cells were not killed by MS2 in the plaques. Such colonies could not be observed when *E. coli* AB259 was used instead of *P. mirabilis* AC2636.

One-step growth experiments. The time course of the growth of MS2 on *P. mirabilis* AC2636 was followed by one-step growth experiments. The adsorption procedure was carried out at 37 C for 5 min in a minimal liquid medium without glucose, at a multiplicity of 0.2. After removing free phages by incubation with anti-MS2 serum for another 5 min, the mixture was diluted 2×10^4 times in prewarmed broth and kept at 37 C to allow the multiplication of phage. Samples were taken at various times and plated before and after treatment with chloroform plus lysozyme. The results, shown in Fig. 1A, indicate that the average burst size of MS2 on *P. mirabilis* AC2636 is 2×10^3, and that the latent period is 45 min. A control experiment was carried out with *P. mirabilis* AC2632, the female parent strain of AC2636; neither adsorption nor phage growth was observed. With *E. coli* AB1528, the male parent strain of AC2636, a burst size of 4×10^3 and latent period of 35 min were obtained (Fig. 1B).

As shown in Fig. 1A, about 10% of the infective centers were resistant to chloroform, which seems to indicate that in 10% of the infective centers MS2 attached to cells of AC2636 without infection, and that this male-specific reversible attachment protected the antigenic site of MS2 from the action of antiserum. A similar situation has been described by Zinder (1963) in the case of phage f2 attached to *E. coli*. When MS2 was adsorbed to *E. coli* AB1528 at a multiplicity of 0.2, about 0.1% of the infective centers were resistant to chloroform.

The origin of the plaques with a colony at the center as described above might be explained by such detachment of the adsorbed phage from male *P. mirabilis* cells and by the low efficiency of adsorption of the phage to the bacteria.

Single-burst experiments. As the determination of the number of *P. mirabilis* AC2636 cells infected with MS2 was not very accurate because of the chloroform-resistant infective centers, the burst size was measured by means of single-burst experiments. A log-phase culture of AC2636 was mixed with MS2 in broth at 37 C for 15 min at a multiplicity of 670. After removal of free phages by incubation with anti-MS2 serum for another 5 min, the mixture was diluted in broth and distributed into 99 tubes, which were incubated at 37 C for 80 min. After treatment with chloroform, one-tenth of the volume of the contents of each tube was plated. The results, shown in Table 4, indicate an average burst size of about 3,000.

Another single-burst experiment was carried out with *P. mirabilis* to test whether the growth of MS2 causes the death of the host cell. The adsorption procedure was the same as that described above, except that the multiplicity was 400. After distribution into 99 tubes, the samples were incubated at 37 C for 60 hr. The growth of male cells was checked by spot tests on minimal agar plates with and without isoleucine and

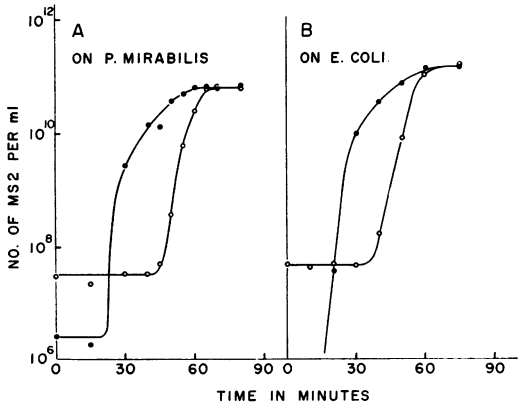

FIG. 1. *One-step growth curves of MS2 on Proteus mirabilis AC2636 (A) and on Escherichia coli AB1528 (B). The titrations were carried out before (○) and after (●) treatment with chloroform plus lysozyme.*

TABLE 4. *Estimation of burst size in single bursts**

No. of tubes†	No. of MS2‡ per tube
89	<10
1	2.2×10^3
1	2.4×10^3
1	2.9×10^3
1	3.0×10^3
1	3.4×10^3
1	3.6×10^3
1	3.8×10^3
1	4.3×10^3
1	5.8×10^3
1	1.06×10^4

* The experimental procedure is given in the text. The average number of infective centers added per tube was 0.15. The average number of male *Proteus mirabilis* cells added per tube was 1.7.

† Total number of tubes, 99; number of tubes containing MS2, 10; average number of bursts per tube, 0.10; number of tubes expected to contain two or more bursts, 1.

‡ Plaque-forming units.

valine. The growth of MS2 was tested by plating one-tenth volume from each tube after treatment with chloroform. MS2 grew in only 2 tubes among 99, and those two did not contain any viable bacteria, indicating that the host cells were killed by growth of MS2 (Table 5).

Absence of host-induced modification and restriction. Bacteriophage λ which has been propagated in *E. coli* K-12 has an exceedingly low efficiency of plating on cells of K-12 which harbor P1 prophage. It has been shown by Arber and Dussoix (1962) that the P1 prophage governs the synthesis of an enzyme which modifies DNA, and a second enzyme which restricts (degrades) all DNA not so modified. A similar modification-restriction system has been shown to operate between *E. coli* B and *E. coli* K-12, so that each modifies and restricts the DNA of the other during conjugational transfer (Boyer, 1964).

A number of observations suggest that the RNA phage, MS2, is not subjected to either restriction or modification. In the first place, the large burst size of MS2 in *P. mirabilis* (over 3,000) makes it unlikely that a significant fraction of the particles have been modified, since the assays are carried out by plating on *E. coli*. Secondly, the data shown in Table 3 suggest that the efficiencies of infection of *P. mirabilis* and *E. coli* with MS2 grown on *P. mirabilis* are the same as those with MS2 grown on *E. coli*.

As a further check on this conclusion, lysates of MS2 were prepared by growth on *P. mirabilis* AC2636 and on *E. coli* AB259 which contained the same number of plaque-forming units per milliliter when assayed on *E. coli* AB259. Broth cultures of *P. mirabilis* AC2636 were then infected with various dilutions of these lysates. After incubation at 37 C overnight, they were treated with chloroform and plated on nutrient agar seeded with *E. coli* AB259. In the tubes which received 10 or more plaque-forming units of MS2, the multiplication of the phage was always observed; on the other hand, in the tubes which had received an average of one plaque-forming unit or less, no phage growth was observed, whether the input phage had been grown on *P. mirabilis* or on *E. coli* (Table 6).

TABLE 5. *Death of the host Proteus mirabilis cells in single-burst experiments**

Growth	No. of tubes	
	With growth of MS2	Without growth of MS2
Male cells	0	36
Female cells	0	18
No bacterial growth	2†	43

* The experimental procedure is given in the text. The average numbers of cells added per tube were as follows: infective centers, 0.05; male cells, 0.4; female cells, 0.5.

† The burst sizes were 1.7×10^3 and 2.5×10^3.

TABLE 6. *Effect of inoculum size of MS2 grown in different hosts on the growth in Proteus mirabilis AC2636**

No. of MS2 added per tube†	No. of plaques after growth in *P. mirabilis* AC2636	
	With phage previously grown in *E. coli* AB259	With phage previously grown in *P. mirabilis* AC2636
1.0×10^4	Confluent	Confluent
1.0×10^3	Confluent	Confluent
1.0×10^2	Confluent	Confluent
1.0×10	863	953
1.0	0	0
0.1	0	0
0.01	0	0

* Tubes containing 10 ml of broth were inoculated with 0.1 ml of an overnight broth culture of *P. mirabilis* AC2636 and with 0.1 ml of various dilutions of MS2 lysates prepared by growing either in *E. coli* AB259 or in *P. mirabilis* AC2636, as indicated. After incubation at 37 C overnight, the cultures were treated with chloroform and 0.1-ml samples were plated on *E. coli* AB259 for plaque counts.

† Plaque-forming units as assayed on *Escherichia coli* AB259.

When lysates of MS2 grown on bacterial strains AB2229 (a Hfr strain of *E. coli* K-12), AB2154 (a P1-lysogenic derivative of AB2229), or *P. mirabilis* AC2636 were plated on nutrient agar seeded with either *E. coli* AB2229 or *E. coli* AB2154, no difference was observed in the number of plaques. When samples of each of the three lysates were added to broth cultures of each of the three bacterial strains, there was no difference in the growth rate of MS2. Thus, no evidence was obtained of restriction or modification of MS2 by *E. coli* K-12, by *E. coli* K-12(P1), or by *P. mirabilis*.

DISCUSSION

It is clear from the results described above that MS2, a RNA phage specific for male strains of *E. coli* K-12, can infect and multiply in *P. mirabilis* when the latter harbors an F-genote derived from *E. coli*. This ability to allow the infection of MS2 seems to be specific for the *E. coli* sex factor, for both *E. coli* and *P. mirabilis* strains harboring a different plasmid, P-*lac*, failed to permit MS2 growth. According to

Wohlheiter et al. (1964) the DNA of P-*lac* has a GC content of 50%, but the physicochemical characters of the β-galactosidase produced by bacteria carrying P-*lac* are different from those of the β-galactosidase produced by *E. coli* K-12.

Recently, Brinton et al. (1964) demonstrated with an electron microscope that a male-specific RNA phage (M-12) is adsorbed to the pili of *P. mirabilis* cells which harbor F-*lac*, but not to those of the F^- cells of *P. mirabilis*. This observation is consistent with our present results obtained with MS2.

The burst size of MS2 growing in *P. mirabilis* AC2636 was found to be 2,000 to 3,000 by both one-step growth and single-burst experiments. This value is approximately the same as that found with *E. coli* male strains. The latent period is definitely longer in *P. mirabilis* than in *E. coli*.

The failure of male *P. mirabilis* strains to give plaques of MS2 may be due to the low efficiency of infection shown in Table 3. There have been several reports that certain small bacteriophages can grow and be liberated from the host bacterium without killing the cell (Hofschneider and Preuss, 1963; Hoffmann-Berling, Dürwald, and Beulke, 1963; Hoffmann-Berling and Mazé, 1964). In the present study, however, no evidence was obtained that lysis of the host *P. mirabilis* cells does not occur after the growth of MS2. Rather, the burst size of 3,000 and the results of the single-burst experiment shown in Table 5 seem to suggest that some, if not all, of the host cells are killed under the experimental conditions used.

No evidence was found of host-induced modification or restriction of MS2 by *E. coli* K-12, by *E. coli* K-12 (P1), or by *P. mirabilis*. It will be of interest to know whether modifying or restricting enzymes will be found which affect RNA.

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